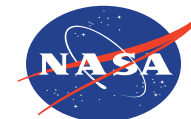




Targeted gene(s) amplification-based NGS analysis of low biomass samples from SAF/Spacecraft surfaces

Nitin K Singh, Jason M. Wood, James N Benardini, and Kasthuri Venkateswaran, 352N,

17th July 2018, Event PPP.4,



Jet Propulsion Laboratory
California Institute of Technology

How to prevent biases in your data analysis?



**IF YOU TORTURE THE DATA LONG ENOUGH,
IT WILL CONFESS TO ANYTHING.**

- RONALD COASE -

Introduction

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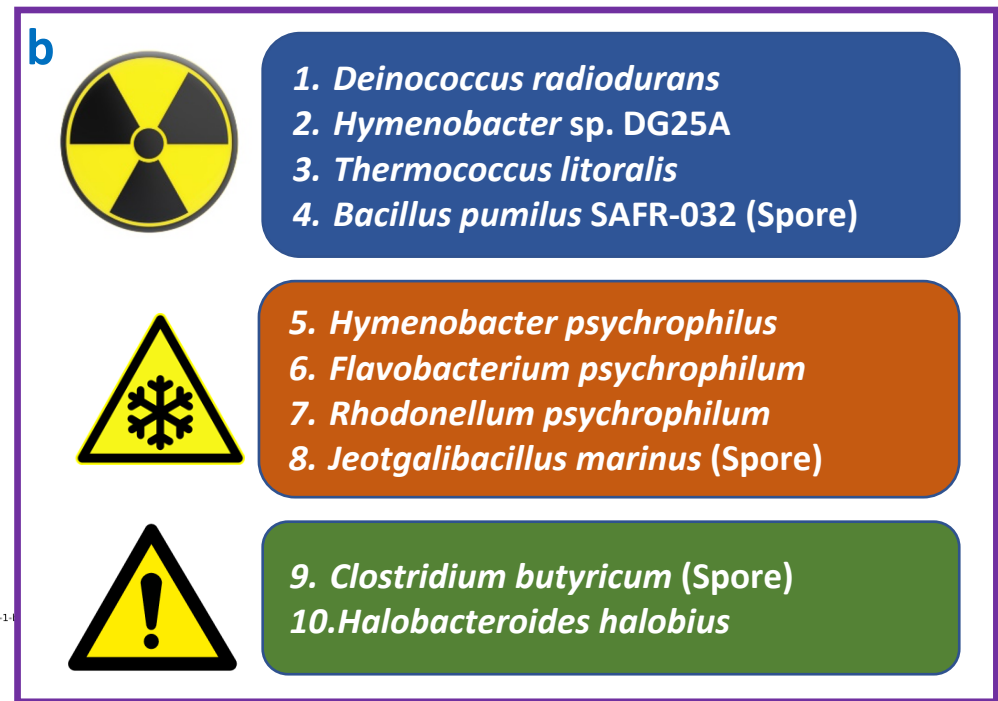
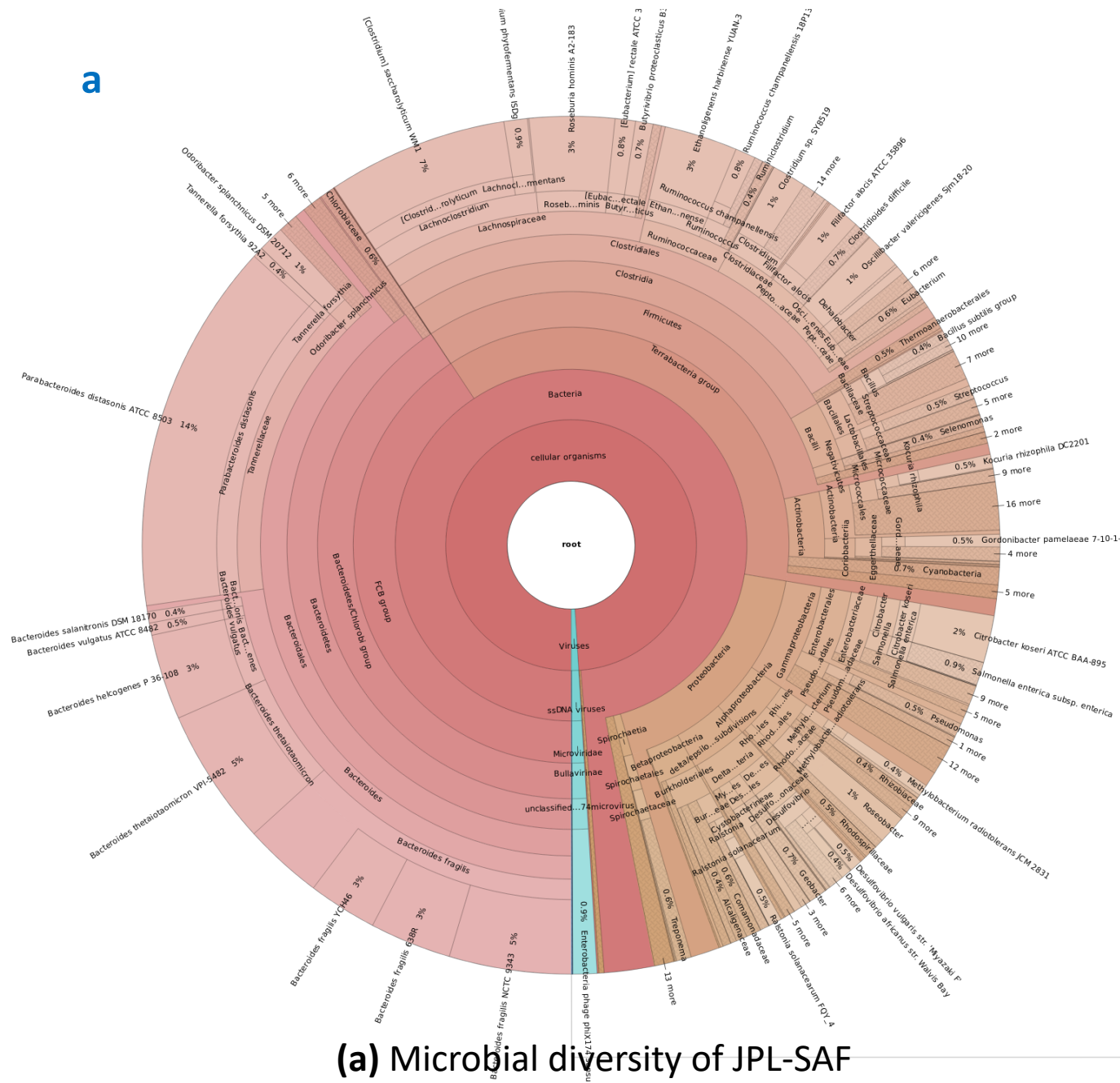


Limiting factors for studying low biomass SAF/Spacecraft surfaces

Conventional technologies are focused on eradicating microbial contaminants, but it is important to understand that low biomass samples contain microbial signatures.

These signature from both Eukaryotic and Prokaryotic domain, need to be cataloged in an unbiased manner to create a microbial passenger list



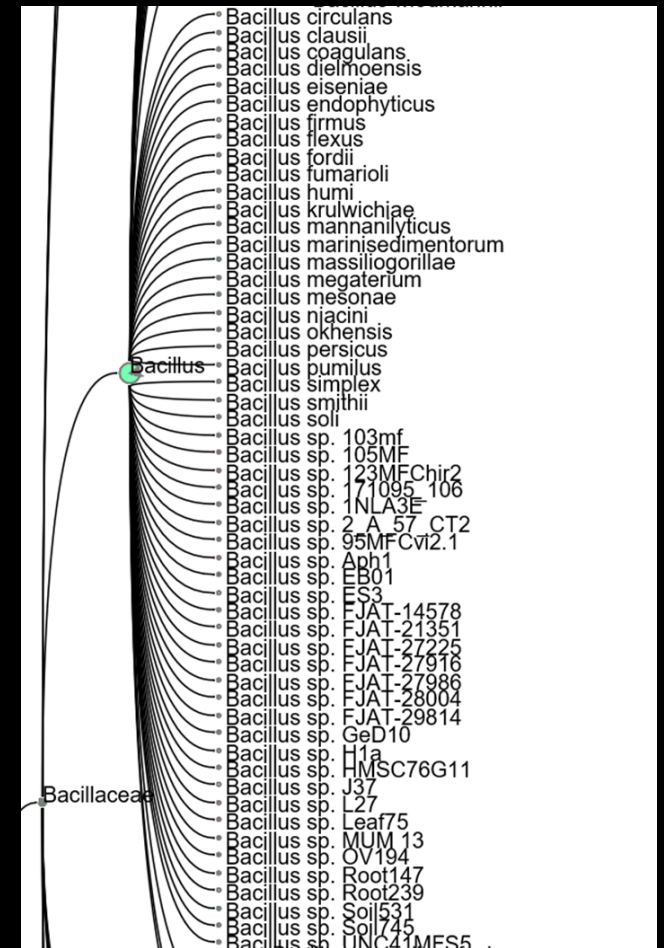
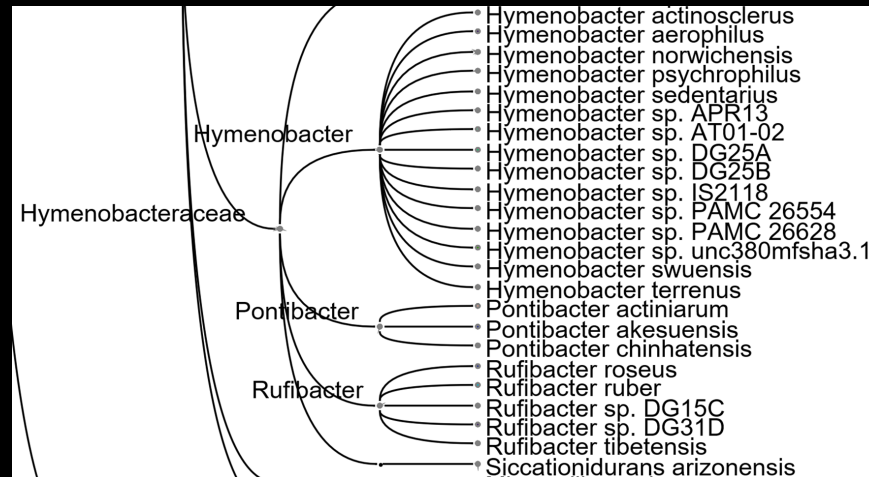
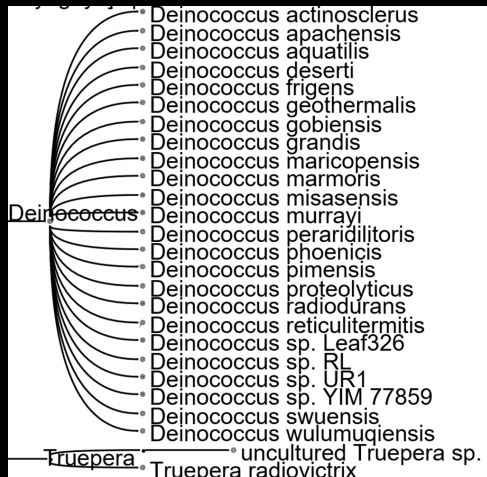


(b) Target microorganisms for Amplicon array.

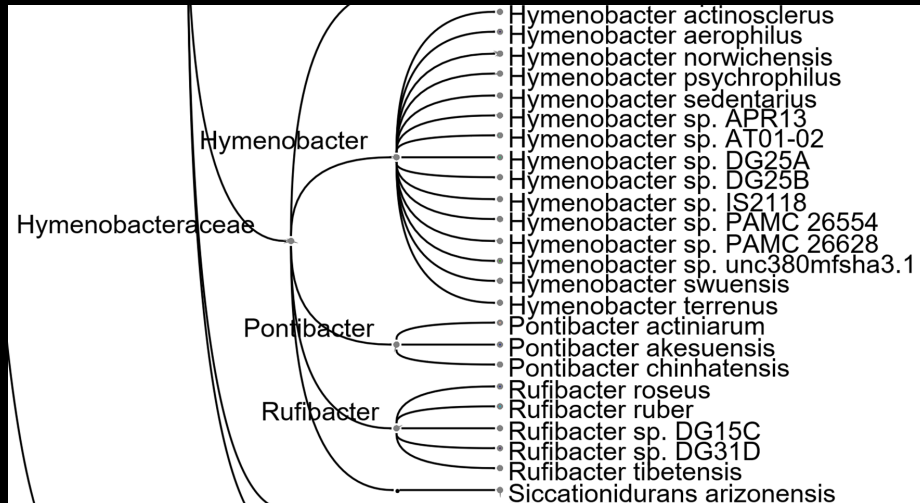
Present status based on 100 sampling events



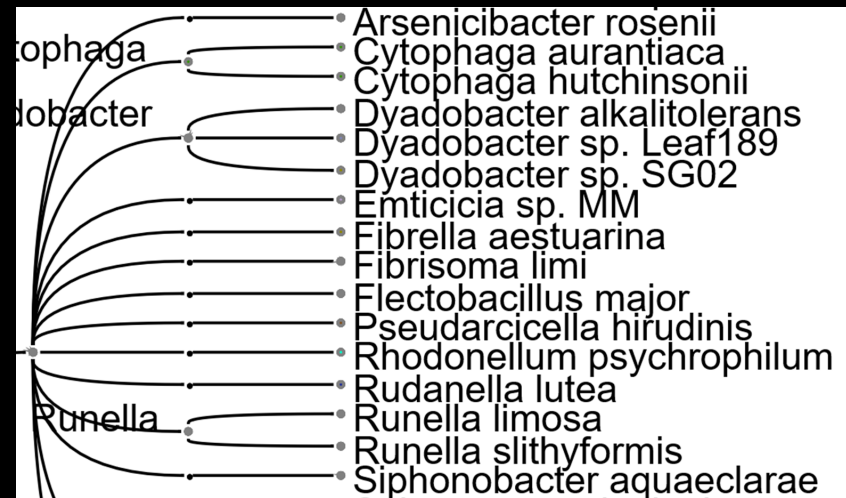
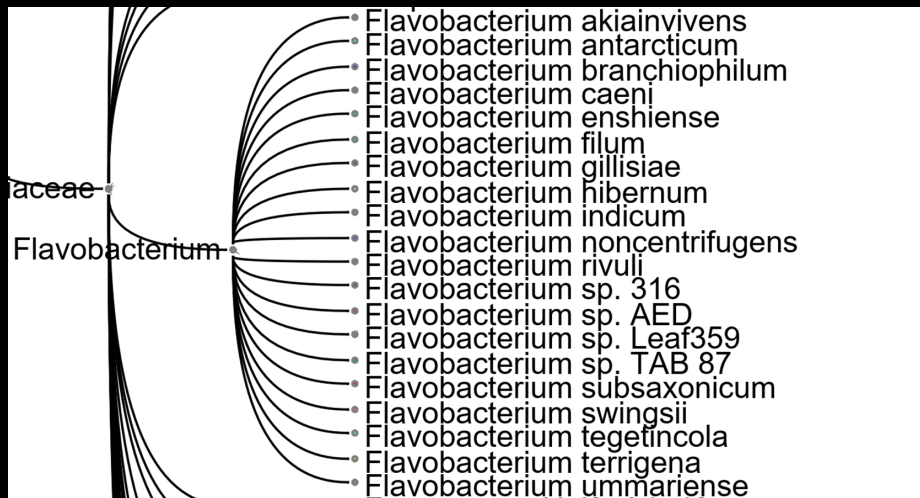
- *Deinococcus radiodurans*
- *Hymenobacter* sp. DG25A
- *Bacillus pumilus* SAFR-032 (Spore)



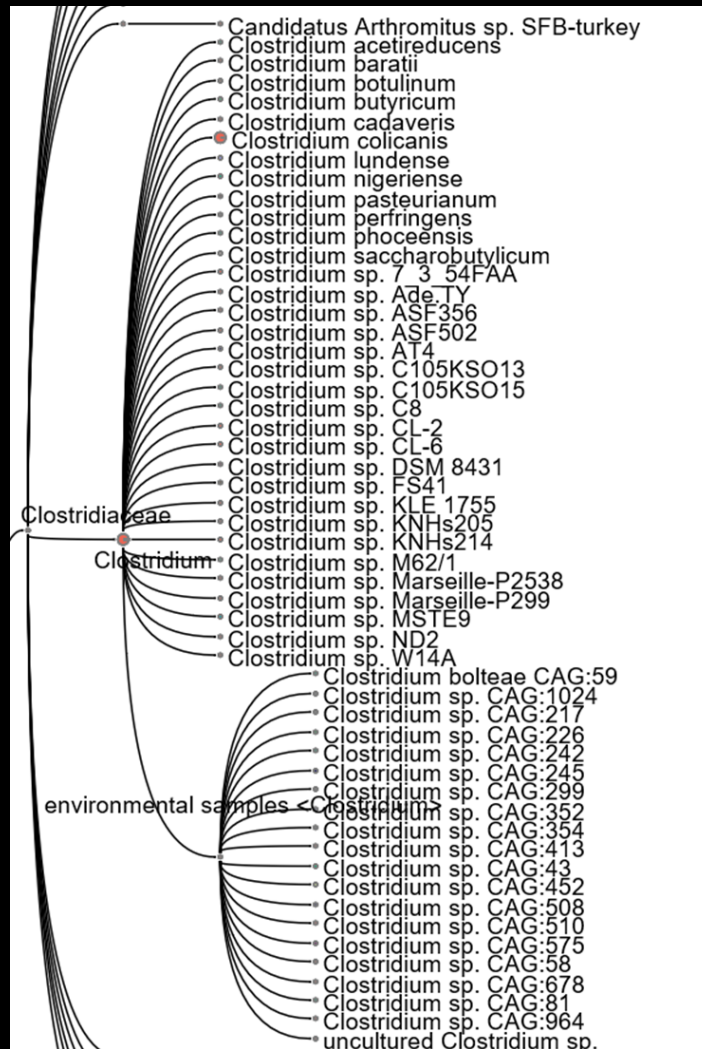
Present status based on 100 sampling events



- *Hymenobacter psychrophilus*
- *Flavobacterium antarcticum*
- *Rhodonellum psychrophilum*



Present status based on 100 sampling events



- *Clostridium butyricum* (Spore)

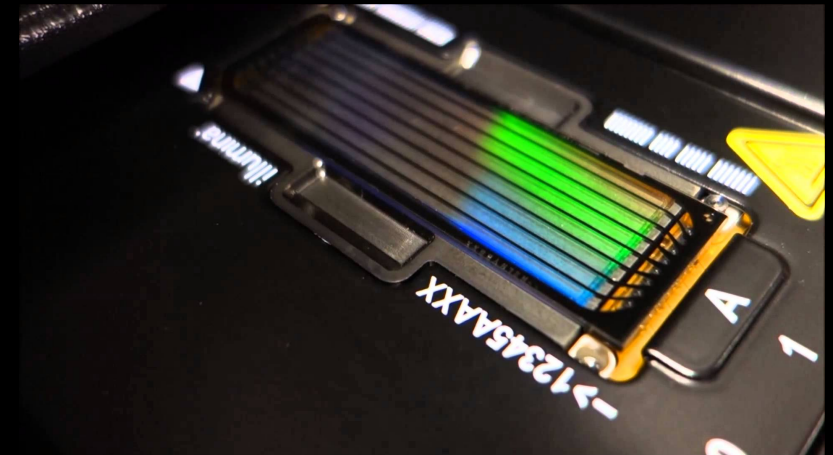
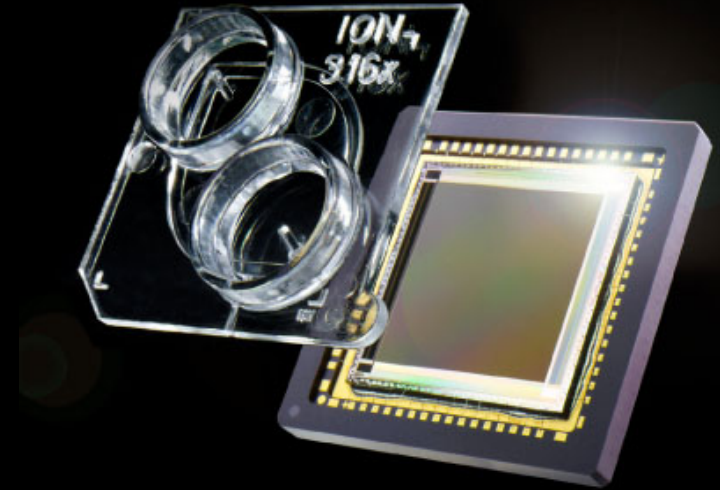
Why use amplicon based technologies for SAF/Spacecraft

- **Targeted gene(s)-based method relies on polymerase chain reaction.**
- **Low biological starting material (<1pg) is enough.**
- **Executable in ultra-low biomass samples such as spacecraft surfaces.**
- **Single panel can be used to study 24,000 targets simultaneously**
- **Applicable for novel species discovery and genetic variation studies**
- **Low computational requirement compared to total metagenome analysis**

Practical application of amplicon based technologies for SAF/Spacecraft

The amplicon based next generation sequencing methods like **AmpliSeq™** from Ion Torrent and targeted gene(s) sequencing from **Illumina**, will assist in understanding cross-domain low biomass surface organisms posing a threat of forward contamination.

Enable the development of suitable cleaning/sterilization procedures and enhance PP verification protocols.



AmpliSeq™ flow-through for target selection to data analysis.

SELECT/CUSTOMIZE
TARGET

CONSTRUCT
LIBRARY

PREPARE
TEMPLATE

RUN
SEQUENCE

ANALYZE
DATA

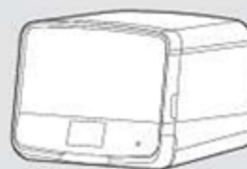
Targeted DNA Sequencing



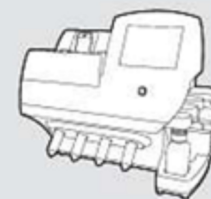
Ion AmpliSeq™ Designer
at Ampliseq.com
Ion AmpliSeq™ DNA Panels



Ion AmpliSeq™
DNA Library Kit



Ion Chef™ System* or
Ion OneTouch™ 2 System



Ion PGM™ Sequencer



Torrent Suite™ Software +
Ion Reporter™ Software

OPEN

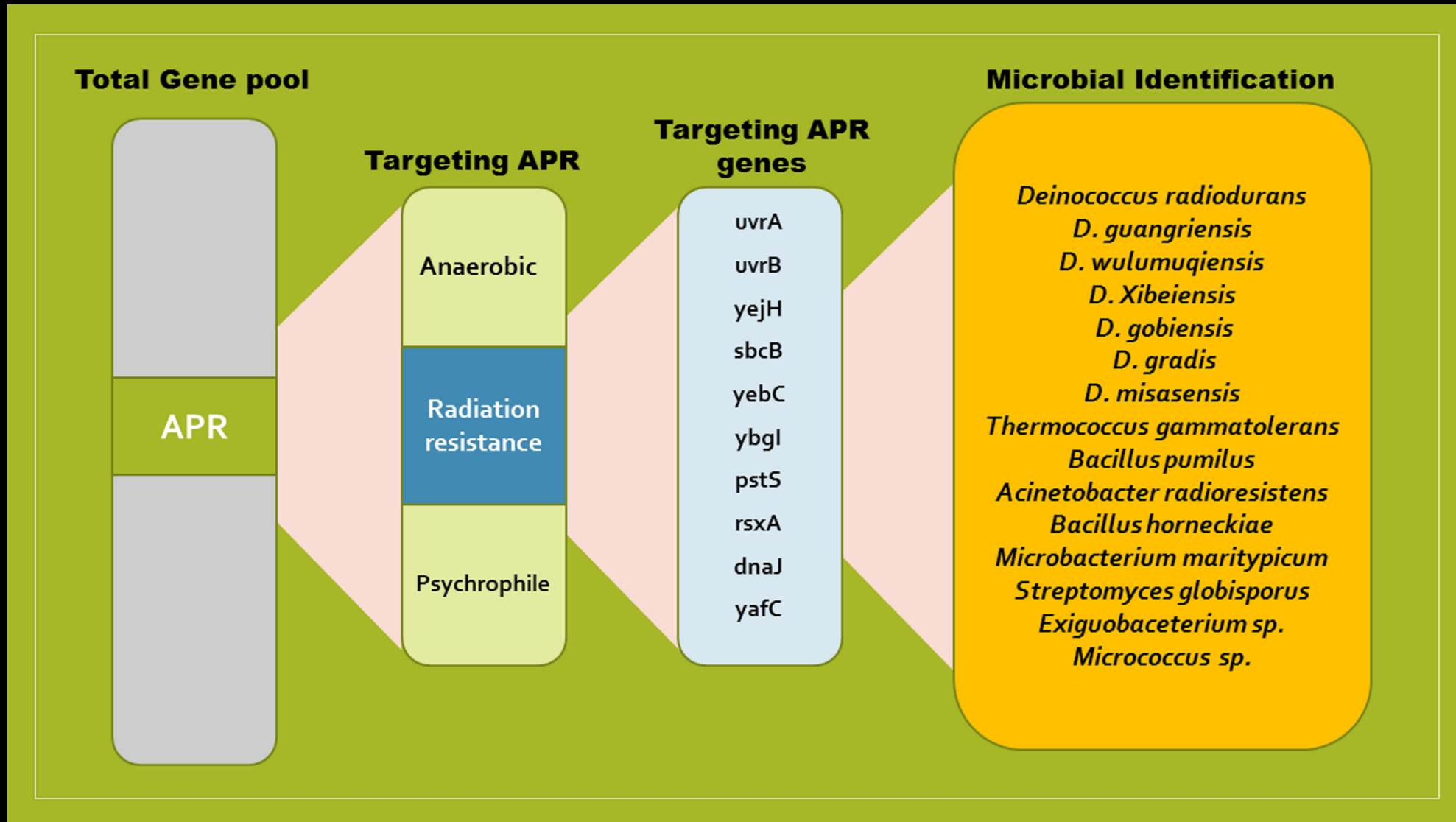
Detection of antimicrobial resistance genes associated with the International Space Station environmental surfaces

Received: 17 August 2017

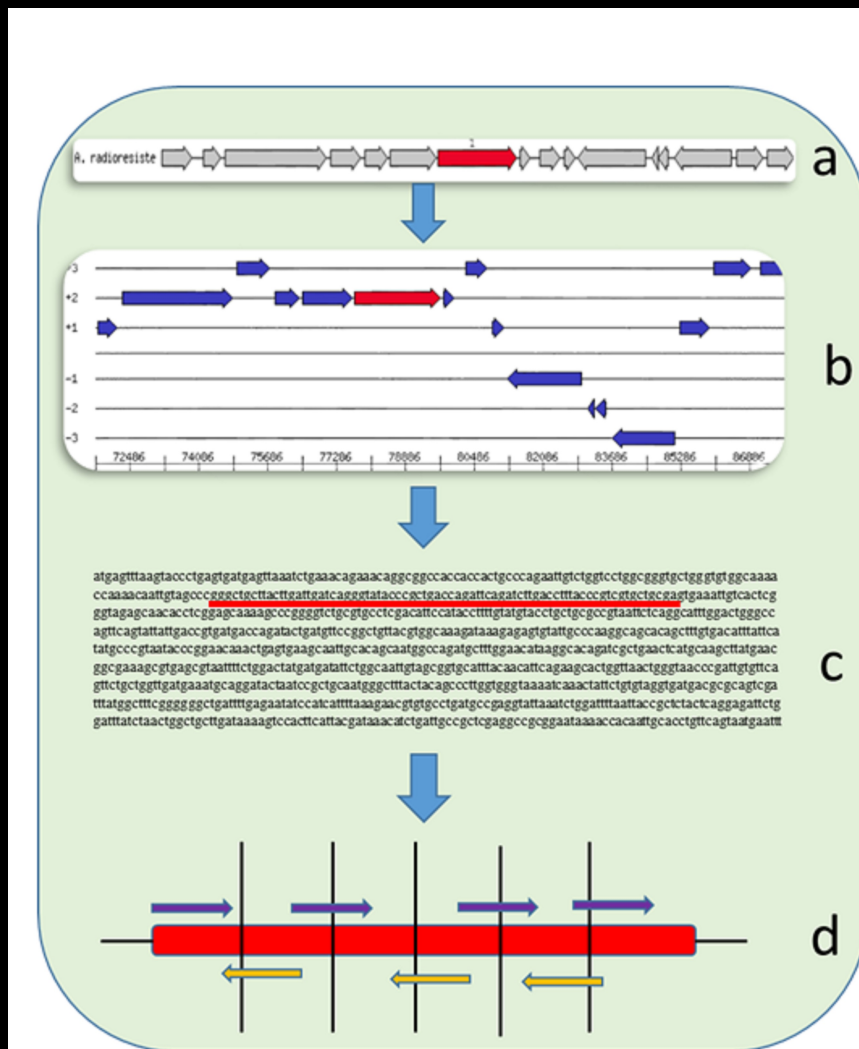
Accepted: 13 December 2017

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Targeting APR genes and organisms



Primer/Panel designing for the APR genes



1. Fragment of the gene of interest from metagenome of a given sample generated (*uvrA*: ~250 bp)

2. Using this as the region, metagenome data mining will be used to identify flanking region(s) and sequence of these flanking regions will yield the genetic information about the gene of interest.

3. Subsequently, the gene of interest will be used for designing multiple primer set(s) for APR AmpliSeq panel.

4. Once sequences are available after APR AmpliSeq, the total sequence of the gene of interest (~1.9-kb for *uvrA* as an example) can be compared for the similarity and hence mutations inferred.

Conclusion

- **Amplicon based technologies will deepen our understanding of microbial population associated with low biomass samples and their functional characteristics with lower cost and computational requirement**
- **Enable cataloging nucleic acid-dependent life surviving extreme conditions of the spacecraft Assembly, Testing, Launch, and Operation (ATLO) environments.**
- **Modulated to catalog the microbial genes of interest to a specific mission (e.g. psychrophilic, anaerobic, radiation resistant microbes for Europa).**

Acknowledgements

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- Edward and Narendra (Jimmy) Patel – supercomputer facility, JPL

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Thanks